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Action of the Ecdysteroid Agonist Tebufenozide in Susceptible and Artificially Selected Beet Armyworm

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Abstract: Toxicity assays with tebufenozide, the first commercial non-steroidal ecdysteroid agonist, against a laboratory strain of the beet armyworm, *Spodoptera exigua* (Hübner), demonstrated the promise of this new compound for the control of this important pest. Experiments to select insects artificially from the laboratory strain by continuous exposure of larval instars to corresponding LC_{25} doses of tebufenozide for over 12 generations ($G_0 \rightarrow G_{12}$: 14–15 months), revealed no loss in susceptibility to the insecticide for up to five generations. Moreover, retention and fate of ^{14}C-labelled tebufenozide were investigated using G_6 larvae from the selection experiments and the results compared with those for the susceptible (G_0) larvae. In addition, piperonyl butoxide, an inhibitor of monooxygenases, when ingested by larvae along with tebufenozide, increased the susceptibility of intoxicated larvae to this ecdysteroid agonist, indicating its oxidative metabolism in *Spodoptera* larvae. © 1998 Society of Chemical Industry.

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Key words: *Spodoptera exigua*; tebufenozide; ecdysteroid agonist; toxicity; resistance

1 INTRODUCTION

The beet armyworm, *Spodoptera exigua* (Hübner), is a polyphagous noctuid of worldwide importance that feeds on various agricultural crops, including vegetables, cotton and ornamentals. During the past decades, the failure of several insecticides such as chlorinated hydrocarbons, organophosphates (OPs) and carbamates to control beet armyworm has been attributed to the development of resistance.[1–5] The extensive use of pyrethroids has also resulted in reduced efficacy in some areas.[6,7] In laboratory studies, tolerance has been documented for the benzoylphenyl ureas (BPUs), diflubenzuron and teflubenzuron.[8,9] In addition, the efficacy of *Bacillus thuringiensis* Berl. formulations against noctuid pests is low or is decreasing. In various beet armyworm populations high levels of resistance to *B. thuringiensis* strains and toxins have been shown in the laboratory.[10]

Tebufenozide [RH-5992; *N-tert*-butyl-*N'*-(4-ethylbenzoyl)-3,5-dimethylbenzohydrazide], a novel synthetic non-steroidal ecdysteroid agonist, which manifests its toxic effects *via* interaction with ecdysteroid receptors, represents a new class of insect growth regulator (IGR).[11–19] Insect larvae treated with

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tebufenozide undergo premature moulting attempts within 24 h after treatment. It has selective toxicity towards lepidopteran pests, and has no adverse effects on mammals, birds, fishes and other vertebrates, and various beneficial insects.[20–24] Because of the development of armyworm resistance to most other insecticides, tebufenozide, with its novel mode of action, is suited for integrated pest (IPM) and insecticide resistance management (IRM) programs for the control of important lepidopteran pests, e.g. *Spodoptera*.

The objectives of this research were to determine the toxicity of tebufenozide against beet armyworm larvae, to determine larval survival after a continuous treatment with sublethal doses of tebufenozide for over one year and to test these larvae for shifts in susceptibility. Moreover, the pharmacokinetics and metabolic fate of tebufenozide were investigated in the initial G_0 susceptible larvae. The synergistic effects of piperonyl butoxide (PBO), a potent inhibitor of oxidative metabolic enzymes, on the toxicity of tebufenozide in beet armyworm larvae were also evaluated.

2 MATERIAL AND METHODS

2.1 Insects

All stages of the beet armyworm were kept at 23($\pm$2)°C, 70($\pm$5)%RH and a 16 : 8 h light : dark photoperiod at the Laboratory of Agrozoology. Larvae were fed a modified Poitout artificial diet; adults were provided with a 10% honeywater solution *ad libitum*.[25]

The susceptible strain originated from a laboratory stock maintained free of pesticide exposure for over 10 years, and was a kind gift from Dr G. Biache (INRA, Guyancourt, France).

2.2 Insecticides

Formulated tebufenozide (240 g active ingredient [AI] litre^{-1} EC) was kindly provided by Rohm and Haas Co. (Spring House, PA, USA) and AgrEvo (Diegem, Belgium). Radiolabelled [*tert-butyl*-^{14}C]tebufenozide (spec. act. 23·06 mCi g^{-1}) was provided by Rohm and Haas Co. The labelled compound was diluted in methanol and kept at $-$20°C.

2.3 Bioassays

2.3.1 Larvicidal tests

Toxicity assays were performed with newly moulted (0–6 h after ecdysis) last (L_5)-instar larvae.[26] One millilitre of freshly prepared modified Poitout artificial diet was dispensed into 2-cm^2 cylindrical wells of 24-well Castor tissue culture plates. A minimum of seven different concentrations of tebufenozide was prepared in distilled water, and 50 µl were uniformly applied to the surface of the solidified diet in each well. Larvae were individually placed on the diet, and 24 larvae were used per dose of tebufenozide. Mortality counts were made at six days after treatment. After this period, control larvae had metamorphosed into one-day-old pupae. Mortality percentages were subjected to probit analysis using POLO-PC program.[27] Toxicity was evaluated on LC_{50} values (95% CI) and slopes($\pm$SE) of estimated toxicity lines, and POLO-PC uses a χ^2 test at $P = 0{\cdot}05$ to detect differences.

2.3.2 Selection assay

Insects with decreased susceptibility were selected from the laboratory susceptible strain of beet armyworm by continuous exposure of all larval instars of each generation for over 12 generations (14–15 months) to tebufenozide. This experiment was started with approximately 10 000 first-instar larvae. These specimens were offered artificial diet that was treated with tebufenozide at the corresponding sublethal LC_{25} dose for different generations (G_{0-5} : 0·5 mg AI litre^{-1}, G_{6-10} : 1 mg AI litre^{-1}; G_{11-12} : 2 mg AI litre^{-1}). This represents a 'worst case' situation of insecticide pressure. Adults were fed untreated honeywater.

At different times during the selection, shifts in susceptibility were calculated by dividing LC_{50} values of larvae under selection by the LC_{50} value of larvae from the starting G_0 generation. Toxicity of tebufenozide towards last-instar larvae was determined as described above. In addition, oviposition of surviving adults was scored as previously described.[25] Pupae were sexed and at least two groups of three couples of newly emerged adults (sex ratio 1 : 1) were kept in a plastic container (11 × 11 × 16 cm) with the inside walls covered with paper to provide oviposition places. The total cumulative number of eggs deposited per female was expressed as a percentage of the mean number of the control groups (433($\pm$51)).

2.3.3 Synergism assay

Technical piperonyl butoxide (PBO) (Fluka, Bornem, Belgium) was tested as a synergist of tebufenozide. The synergist solution was prepared at a ratio of 1 : 5 (tebufenozide : PBO). This treatment included two replicated tests with 480 newly moulted (0–12 h) last-instar larvae each, and tebufenozide was simultaneously provided alone or with PBO. No mortality was scored when last-instar larvae of *S. exigua* were fed PBO up to the highest concentration used (5 mg litre^{-1}). Percentages of mortality were corrected according to Abbott for untreated mortality,[28] and results analysed with the probit option of POLO-PC.[27] A synergism ratio was calculated by dividing the LC_{50} for tebufenozide alone by that obtained with the tebufenozide : PBO mixture.

2.4 Fate of tebufenozide

Newly moulted (0–2 h) last-instar larvae were selected and individually starved for 6 h in a 4·5-cm plastic Petri dish. Two microlitres of methanol containing about 200 000 dpm [^{14}C]tebufenozide was applied on a 5-mm-diameter disc of a freshly cut castor bean leaf (*Ricinus communis* L.).[29] After solvent evaporation, one disc was offered to one last-instar larva in a glass Petri dish of 30 mm diameter. Larvae that had not completely consumed the leaf disc were removed from the assay. Three replicates of two last-instar larvae were selected after 2 and 6 h of ingestion of the leaf disc, and stored in the freezer at −20°C until analysis.

For dissection, larvae were ligated at the oral and anal ends with dental wax thread to avoid any extrusion of ingested contents during collection of haemolymph and dissection of gut and integument (carcass). After dissection of the different body tissues in ice-cold physiological solution (Grace's insect tissue culture medium, Sigma, Bornem), these parts were rinsed and quickly blotted on tissue paper. Radioactivity was extracted by homogenizing the tissues in methanol + water (9 + 1 by volume; 3 ml) in 1% acetic acid on ice with a tissue electrohomogenizer (Heidolph, Germany) for 10 s. After homogenization, samples were centrifuged at 10 000*g* for 10 min at 4°C. The supernatant was collected and kept on ice. The pellet was re-extracted with extraction solvent by rigorous vortexing and centrifugation. The two supernatants were pooled and lyophilised (Savant, Germany). The lyophilised samples containing extracted radioactivity from the different tissues were then diluted in 200 µl methanol and filtered using a 0·2-µm filter (13 mm diameter; Acrodisc LC13 PVDF, USA). The radioactivity in a 5-µl sample of each extract in 5 ml of 'Radio-Safe TM' (Beckman, CA, USA) was measured using a Beckman LS-6500 Multi-purpose scintillation counter (CA, USA).

3 RESULTS

3.1 Larvicidal toxicity

Larvae intoxicated with tebufenozide showed apparent signs of precocious and lethal moulting within 12–24 h of treatment. The head capsule slipped down, revealing a double head capsule, and a fragile and non-sclerotized new head capsule was observed underneath the old capsule. Simultaneously, feeding and weight gain of such treated larvae were significantly suppressed (data not shown).

3.2 Selection assay

3.2.1 Shift in toxicity

For last-instar *S. exigua* larvae of the susceptible stock (G_0) fed on treated artificial diet, an LC_{50} of 0·60 mg AI litre^{-1} (0·56–0·65) was calculated; the toxicity curve slope was 13·1(±2·2) (Table 1). Subsequent selection by continuous treatment with a corresponding LC_{25} of 0·5 mg AI litre^{-1} tebufenozide over the first five generations did not result in a shift in susceptibility to tebufenozide (Table 1, Fig. 1). Although, after four generations of continuous treatment, the LC_{50} against last-instar larvae was 0·69 mg AI litre^{-1}, and reached 1·19 mg AI litre^{-1} in the fifth generation, this difference was not statistically significant at $P = 0{\cdot}05$ (χ^2 test). In addition, χ^2 test for parallelism confirmed that the slopes of the toxicity lines were not significantly ($P = 0{\cdot}05$) different. Further selection resulted in last-instar larvae of the sixth generation with a 4-fold decrease in susceptibility as compared to G_0 larvae, since the LC_{50} was 2·61 mg AI litre^{-1}. Moreover, the slope of the toxicity line was significantly lowered: 3·6(±0·5) resulting in a significant resistance ratio at LC_{90} of 7·8. Continued selection

TABLE 1
Selection Assay in the Susceptible Strain of *Spodoptera exigua*[a]

Generation	LC_{50} (*95% CI*) (*mg AI litre*$^{-1}$)[b]	*Ratio*[c]	*Slope*(±*SE*)	LC_{90} (*95% CI*) (*mg AI litre*$^{-1}$)[b]	*Ratio*[c]
G_0	0·60 (0·56–0·65)a	1	13·1 (±2·2)a	0·75 (0·70–0·85)a	1
G_4	0·69 (0·52–0·87)a	1·1	12·5 (±1·7)a	0·87 (0·66–1·39)a	1·2
G_5	1·19 (0·75–1·56)a	1·9	9·9 (±2·0)a	1·58 (1·18–2·55)a	2·1
G_6	2·61 (1·86–3·51)b	4·4	3·6 (±0·5)b	5·87 (4·26–10·12)b	7·8
G_7	2·90 (1·73–3·77)b	4·8	3·8 (±0·8)b	6·52 (4·39–9·12)b	9·3
G_8	2·99 (1·89–3·99)b	5·0	3·1 (±0·9)b	7·14 (4·45–11·77)b	9·5
G_{10}	3·10 (1·79–4·12)b	5·2	3·0 (±0·9)b	7·66 (5·35–12·76)b	10·2

[a] Induction of tolerance *via* continuous treatment of sublethal doses of tebufenozide (~LC_{25}) over subsequent generations *via* administration to all five larval stages of a susceptible laboratory strain.
[b] Toxicity is expressed as LC_{50} and LC_{90} (mg AI litre^{-1}) based on mortality percentages scored at ecdysis in controls plus 24 h (=6 days after start of treatment); data are based on a minimum of seven different doses and 24 newly moulted (0–12 h old) last-instar (L_5) larvae per dose. Data followed by a different letter (a–b) within the same column are significantly different at $P = 0{\cdot}05$ (χ^2 test).
[c] Tolerance ratio of the laboratory strain; LC_x selected strain/LC_x initial susceptible strain (G_0).

pressure with tebufenozide of five subsequent generations over six months did not increase ($P = 0{\cdot}05$) the LC_{50} values nor result in a further shift in the slopes for toxicity (Table 1, Fig. 1). It should be noted that, from the sixth generation until the end of the assay, the resistance factor at LC_{90} ranged between 7·8 and 10·2; however, no significant differences were calculated. Furthermore, the selected colony of *S. exigua* did not survive beyond the twelfth generation because of a loss in oviposition.

3.2.2 *Effects on fecundity*

During the selection assay, especially from the fourth generation onwards, oviposition was conspicuously reduced to 65(±8)% of oviposition by the initial G_0 population adults (Fig. 2A). Two generations later (G_6), oviposition of surviving adults had fallen to 48(±9)%. Furthermore, egg-laying of the surviving adults of the tenth and eleventh generation was only 24(±6)% and 11(±5)%, respectively. Finally, the twelfth generation was lost because the eclosed adults did not oviposit any eggs. A regression plot of the data obtained showed a clear negative relationship between susceptibility, expressed as LC_{50}, and fecundity, as the mean percentage of eggs per female as compared to controls (Fig. 2B).

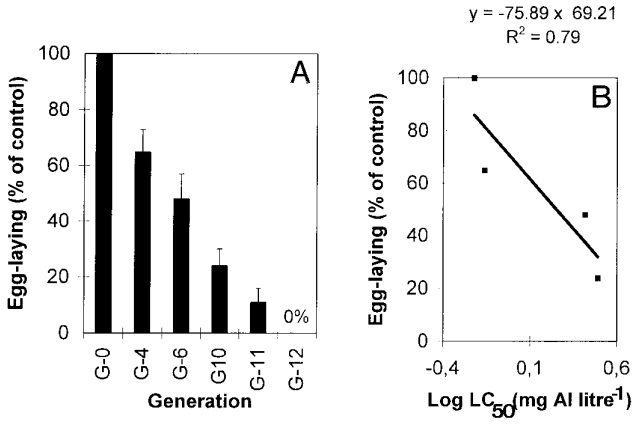

Fig. 2. A. Effects on egg-laying of surviving adults of *Spodoptera exigua* during selection assay with tebufenozide. Data are expressed as mean percentages (bar ± SE) of the number of eggs per female of G_x as compared with the initial population (G_0). B. Linear regression plot showing the negative relationship between the mean fecundity, expressed as percentage of the number of eggs per female of the G_x generation as compared with G_0 moths, and the logarithm of LC_{50} for tebufenozide.

3.3 Synergism by PBO

In order to determine the synergistic effects of PBO in increasing the toxicity of tebufenozide in *S. exigua*, larvae, thus evaluating the possible involvement of mixed function oxidases, G_0 larvae were fed on artificial diet containing a mixture of tebufenozide and PBO at five times the concentration of tebufenozide. Feeding of *S. exigua* with artificial diet treated with PBO up to a concentration of 5 mg litre^{-1} resulted in no mortality. This dose was five times higher than the doses used to verify the effects on tebufenozide. The addition of PBO enhanced the biological effect of tebufenozide to a great extent, resulting in an LC_{50} of 0·18(0·14–0·21) mg litre^{-1} as compared to an LC_{50} of 0·58(0·52–0·64) mg litre^{-1} with tebufenozide alone (Fig. 3). The calculated synergism ratio was 3·4. Typically, the toxicity line was

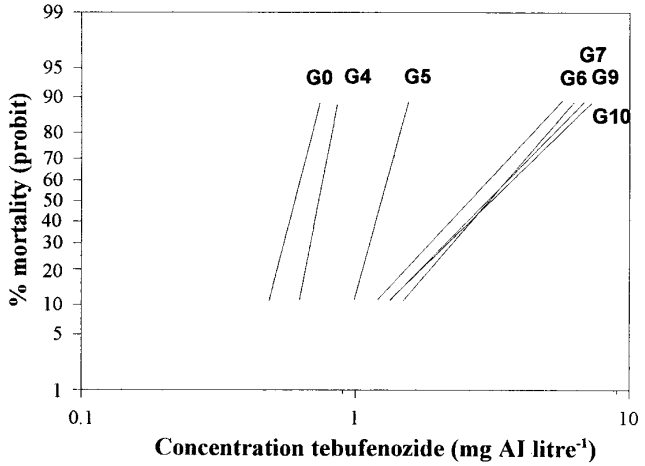

Fig. 1. Selection assay in the susceptible strain of *Spodoptera exigua*. Toxicity of tebufenozide on last-instar larvae of different generations after continuous treatment with tebufenozide. Treatment by uniformly covering the surface of artificial diet with formulated tebufenozide. Mortality percentages were scored after six days, and the data used to calculate LC_{50}s (95% CI) by probit analysis.

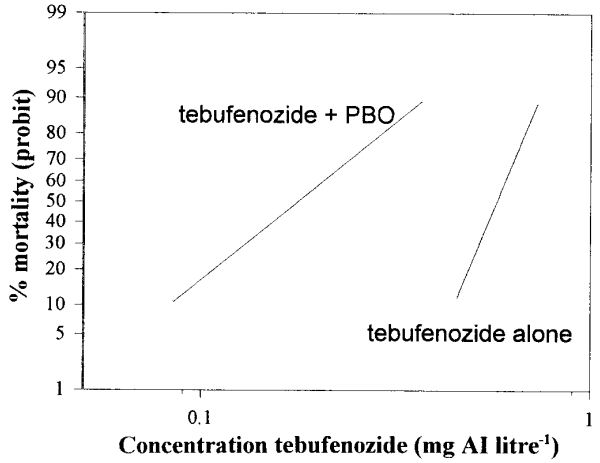

Fig. 3. Effect of piperonyl butoxide on the toxicity of tebufenozide to last-instar larvae of *Spodoptera exigua*. The ratio tebufenozide : piperonyl butoxide was 1 : 5. The treatment was executed by uniformly spreading tebufenozide with or without PBO onto the surface of artificial diet. Mortality percentages were scored after six days, and LC_{50}s (95% CI) calculated by probit analysis.

significantly flattened by the addition of PBO; the slope(±SE) reached 4·43(±0·50) for tebufenozide + PBO whereas it was 11·68(±1·73) for tebufenozide alone.

In a second assay, last-instar larvae of the tenth and eleventh generation of the selection assay were fed 0·5 mg litre^{-1} tebufenozide in mixture with 2·5 mg litre^{-1} PBO, resulting in 71% mortality. Another group was offered diet treated with 0·5 mg litre^{-1} tebufenozide only and 12% mortality was scored, leading to a synergism activity of 6-fold.

3.4 Fate of tebufenozide

At 2 h after uptake, higher levels of radioactivity (mean(±SD)) were recovered in the three body tissues, haemolymph (11(±2)%), carcass (15(±2)%) and gut (35(±5)%) of susceptible larvae (G_0) as compared to G_6 last-instar larvae (6(±1)%, 10(±4)% and 29(±3)%, respectively). As a consequence, the excreta of G_6 last-instar larvae had higher amounts of radioactivity. Four hours later, the levels of radioactivity in the G_6 larval body tissues (haemolymph: 1(±1)%, carcass: 2(±1)% and gut: 4(±1)%) were apparently lower compared to susceptible strains (6(±3)%, 7(±2)% and 17(±4)%, respectively). In the excreta of G_6-larvae, 93(±2)% of the total recovered radioactivity was recorded, while in susceptible species this percentage was only 70(±8)%.

4 DISCUSSION

The most salient effect of tebufenozide in last-instar larvae of *S. exigua* is precocious moulting induction and growth inhibition, resulting in its toxicity. This is consistent with observations in other insects.[12–16,25,30] Similar results of death during a prematurely induced moulting have been reported following application of natural ecdysteroids,[31,32] indicating a state of hyperecdysonism (in the sense of Williams[33]). This confirms the moulting hormone-mimicking action of tebufenozide.

It was promising that *S. exigua* larvae continuously exposed to LC_{25} concentrations of tebufenozide for five generations, representing a period of six months, displayed a very low potential for resistance development. Similarly, previous attempts at selection of tolerance to tebufenozide over four generations in the cotton leafworm, *Spodoptera littoralis* (Boisduval) were not successful.[28] In contrast, Van Laecke[8] reported a 2- to 3-fold tolerance to the BPU teflubenzuron in *S. exigua* already after four generations with only a single treatment with the insecticide during the second larval instar. With tebufenozide, further selection over 14–15 months resulted in only a 4- to 5-fold decrease in toxicity. In this context, Brown & Pal[34] stated that at the beginning of a selection process, a slight increase in LD_{50} values may be independent of specific genes for resistance. Thus, weaker individuals become eliminated in the early generation(s) of selection and the stronger specimens, being more fit and showing increased vigour, survive. In addition, it should be remarked that, at the end of the assay, the resistance factor at LC_{90} reached around 10. This could be important; however, there is no simple relationship between the severity of resistance and the resistance factor and it may vary with species, compound and assay type. Albeit, we think that this level of loss in toxicity is still within the range of susceptibility, but further analysis is required, especially with strains collected in areas with severe control problems.

In addition, the current observations during the selection assay raise the suggestion that selection for tolerance to tebufenozide may be linked with fitness factors such as egg-laying of surviving specimens. Although our results should be interpreted cautiously, they suggest the idea of a fitness cost associated with insecticide resistance to tebufenozide. However, we have no mechanistic explanation for the surprising negative association between resistance and fecundity. We may hypothesize an association with enhanced metabolic detoxification of the toxophore. For instance, resistance to OPs in the cotton aphid, *Aphis gossypii* Glover, has been associated with high carboxylesterase activity, and elevated concentrations of esterases were often associated with fitness costs.[35,36] In addition, we found a limitation of our assay, in that we only measured one component of fitness, egg-laying. Although fecundity is a readily measurable and important component of fitness, it is not necessarily an accurate indicator of overall fitness. As such, we cannot exclude the hypothesis that changes in other fitness components compensated for the decrease in fecundity associated with tolerance to tebufenozide. Altogether, the current finding may be of practical use for the prevention of resistance in the field, although more data are needed to prove this hypothesis.

The fate of substituted dibenzoylhydrazines like RH-5849 and tebufenozide in other insects has been studied in some detail.[29,37] The insecticides show high metabolic stability and are excreted rather quickly as parent compound in different armyworms and *Leptinotarsa decemlineata* Say beetles after absorption in the body tissues. Further, the current data on pharmacokinetics of labelled residues of [^{14}C]tebufenozide in susceptible and the less susceptible G_6 beet armyworm larvae suggested that absorption of radiolabelled residues from the gut into the body haemolymph is either somewhat lower in G_6 larvae or it is more rapidly absorbed back in the rectum and eliminated along with the excreta. In addition, our data show that the rate of immediate passage and elimination *via* the gut without uptake in the body haemocoel, might have been increased after a continuous selection treatment over six generations.

In addition, we hypothesize that tebufenozide molecules show a high metabolic stability in the insect

body. Previous TLC profiles of fractionated radioactivity revealed that most (90–95%) radioactivity recovered in susceptible caterpillar body tissues consisted of original tebufenozide.[29] It is well known that the gut is a primary source of degradative enzymes against insecticides, and we have found that a higher amount of parent toxophore was converted into metabolites in the gut of selected larvae, resulting in less original tebufenozide than with susceptible strains (unpublished results). Such a higher breakdown activity might lead to lower levels of the parent toxophore and, secondly, metabolism may result in a differential pattern of uptake in the body tissues and excretion. However, further experiments are required before reaching definite conclusions. Although, up to now, the data may strengthen the notion that tebufenozide is metabolized to a somewhat higher extent in the gut of larvae that show a somewhat lower susceptibility. In addition, the metabolites formed are most probably eliminated from the gut in a more rapid manner without being absorbed in the body haemocoel; this was more apparent in larvae that showed a lower susceptibility as compared to susceptible specimens. Similarly, Smagghe *et al.*[38] reported that, in a laboratory strain of *S. littoralis*, about 90% of the amount of radioactivity in the whole body consisted of parent tebufenozide, whereas this was only 55% in a field strain, suggesting that different toxicities of tebufenozide in different strains may be attributed to different rates of metabolism of the compound.

Increased metabolic activity in insects is an important sign of tolerance/resistance to various compounds. The synergistic toxic effect of PBO, an inhibitor of enzymes for oxidative metabolism, with tebufenozide therefore suggested that oxidases are important in the detoxification of tebufenozide. This agrees with the findings of Thirugnanam[39] who demonstrated increased efficacy of tebufenozide with other P_{450}-enzyme inhibitor compounds. Likewise, our own unpublished HPLC data show the production of alcoholic, ketone and aldehyde metabolites of tebufenozide, as a result of oxidation of the alkyl substituents of the two aromatic benzoyl rings. As such, we hypothesize that the major first-phase route for tebufenozide detoxification is through oxidation. Furthermore, polar and very polar metabolites which are inactive components are formed. Cleavage between the carbonyl and amide moiety to result in hydrolytic products could not be identified; however, this process cannot be excluded. The option that hydrolysis is of minor importance agrees with preliminary results showing that addition of DEF (*S,S,S*-tributylphosphorotrithioate; Celamerck, Ingelheim, Germany), a potent hydrolase inhibitor, only enhanced the toxicity of tebufenozide by 1·5-fold (G. Smagghe, unpublished data).

The current laboratory data and the toxicity scores under semi-field conditions by Smagghe and Degheele[40] together with the laboratory and field settings of Chandler[41] are true indicators that the activity of tebufenozide shows promise for practical application against armyworm larvae. This agrees with previous experiments where tebufenozide was shown to be highly toxic against various other lepidopteran larvae, both by feeding treated leaves or artificial diet and after topical application.[25,30] In addition, the calculated toxicity with the beet armyworm strains agrees with other results in field and laboratory strains of *S. littoralis*. Ishaaya *et al.*[42] found that an Israeli field strain, that was over 100-fold resistant to OPs and pyrethroids, showed a 3-fold higher LC_{50} for tebufenozide as compared to a laboratory strain. More recent, a field/laboratory F/L)-ratio of 4 was calculated for tebufenozide in multi-resistant field strains of *S. littoralis*, whereas for chlorpyrifos that ratio reached 30 and for carbamates >200.[28] In addition, Sauphanor and Bouvier[43] reported on resistance and cross-resistance to BPUs and benzoylhydrazine when comparing a laboratory susceptible population of the codling moth, *Cydia pomonella* L., with (multi-resistant) field-strain larvae from southern France. Their tests revealed >370-fold resistance to diflubenzuron, whereas for tebufenozide a 26-fold lower susceptibility was recorded. The F/L-ratio for tebufenozide in the F_1 population varied around 13. However, a recent study by Regiroli and Manaresi[44] demonstrated lack of cross-resistance between dimilin and tebufenozide in *C. pomonella* larvae resistant to DFB. In addition, the high levels of resistance to OPs, BPUs and fenoxycarb developed by tufted apple bud moth, *Platynota idaeusalis* Walker, populations clearly did not lead to resistance to tebufenozide.[45] As such, the potency of tebufenozide as a tool to control the beet armyworm and as a part of an IRM program may be strengthened. However, futher extended biological and biochemical research on tolerance/resistance and cross-resistance with other currently used IGRs is in progress and will help to provide clear guidelines for growers. Hence, in order to prevent development of resistance, it is recommended that tebufenozide should be alternated with other groups of insecticides.[21]

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